

Behaviour of Endosulfan Residues in Peppers, Cucumbers and Cherry Tomatoes grown in Greenhouse: Evaluation by Decline Curves

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Abstract: Decline of endosulfan residues in pepper, cucumber and cherry tomatoes grown in greenhouses, and the corresponding statistical parameters, were evaluated assessing the formal approaches proposed by Timme, Frehse and Laska to study the behaviour of pesticide residues in crops. In all cases, the first-order reaction function was confirmed to describe the behaviour of endosulfan residues, but functions that best fit the experimental data were first-order for cucumber, RF-first-order for pepper and RF-1.5th-order for tomato. Half-life periods of endosulfan residues determined from the first-order function were $11.1(\pm 1.3)$ days (cucumber), $15.2(\pm 3.3)$ days (pepper) and $20.1(\pm 6.6)$ days (tomato), whereas the values obtained from the optimal functions were close to 10 days for the three plantations.

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1 INTRODUCTION

Endosulfan (Thiodan®, Cyclodan®, Beosit®, Malix®) is a broad-spectrum non-systemic contact and stomach insecticide effective for control of sucking, chewing and boring insects and mites on a very wide range of crops. It is used in many countries throughout the world for the control of pests on fruits, vegetables and tea and on non-food crops such as tobacco and cotton.¹

Technical endosulfan consists of a mixture of α - and β -isomers in the approximate ratio 70 : 30.^{2,3} Both α - and β -endosulfan are fairly resistant to photo-degradation,⁴ but the two dominant breakdown products (endosulfan sulfate and endosulfan diol) are susceptible to photolysis.⁵ In soil and on plant surfaces,

endosulfan sulfate is the primary degradation product of endosulfan.⁶

In recent years, endosulfan residues (expressed as sum of α -endosulfan, β -endosulfan and endosulfan sulfate) were most commonly found by regulatory agencies in residue monitoring^{7–10} and total diet studies.^{8,11,12} In Almería, a Spanish province where about one million metric tons of vegetables are produced annually in greenhouses, endosulfan is widely used, and its residues are regularly monitored by the producers of this area. In the agricultural season of 1993/94, 728 samples of pepper, cucumber and tomato grown in Almería were analysed in the residue monitoring carried out by COEXPHAL (an association of producers and exporters of fruits and vegetables in Almería), and about 37% of these samples were found to contain endosulfan residues.¹³

The objectives of this work were to study the behaviour of endosulfan residues in pepper, cucumber and

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cherry tomatoes grown in commercial greenhouses under the particular climatic conditions of Almería, and to assess the mathematical models proposed by Timme, Frehse and Laska¹⁴ to determine the statistical parameters that describe these processes.

2 EXPERIMENTAL

2.1 Chemicals and apparatus

Standards of endosulfan (α , β and sulfate) were supplied by Riedel-de Haën (Seelze, Germany). All solvents (pesticide residue grade) were obtained from Merck (Darmstadt, Germany). Endosulfan standard solutions were prepared using light petroleum as solvent.

The gas chromatograph was a Perkin Elmer, model 8700, equipped with an electron-capture detector and 5% SE-30 on Chromosorb W HP 80–100 mesh packed column (2 m \times 3 mm ID). The chromatographic conditions used for the analysis of endosulfan residues were: injector 250°C; detector 250°C; oven 200°C; carrier gas (nitrogen) flow rate, 20 ml min⁻¹; auxiliary gas (nitrogen) flow rate, 40 ml min⁻¹.

2.2 Decline studies

Decline experiments were conducted in three commercial greenhouses belonging to Campos de Nijar S.A., located in Nijar, 40 km northeast of Almería. The greenhouse sizes and plantation densities were 0.52 ha, 17 500 plants ha⁻¹ (pepper); 0.55 ha, 16 000 plants ha⁻¹ (cucumber) and 0.38 ha, 20 000 plants ha⁻¹ (tomato).

Plants receiving routine horticultural treatment were sprayed on 28 October 1991, with endosulfan 357 g litre⁻¹ EC at a concentration of 3 ml litre⁻¹ and a spray volume of 845 litre ha⁻¹ (pepper), 770 litre ha⁻¹ (cucumber) and 615 litre ha⁻¹ (tomato).

The average of the daily maximum/minimum temperatures outside and inside the greenhouses throughout the study were 20°C/10°C and 25°C/10°C, respectively, whereas the maximum/minimum absolute temperatures outside and inside the greenhouses were 23°C/3°C and 29°C/5°C, respectively. Average relative humidity outside and inside the greenhouses was 70% and 80% respectively, and average solar irradiation outside and inside the greenhouses were 3 kWh m⁻² day⁻¹ and 2 kWh m⁻² day⁻¹, respectively.

For each vegetable, three samples were collected at random from sampling plots of 200 m² at 1, 3, 7, 14, 22, 32 and 38 days after application. The samples consisted of 15–20 pieces of peppers (160–180 g per piece), 10 pieces of cucumbers (400–500 g per piece) and 60–70 pieces of cherry tomatoes (7–9 g per piece). Immediately after picking the samples were put into polyethylene bags and transported to the laboratory, where they

were chopped, blended, thoroughly mixed and divided into three subsamples each. The subsamples were kept deep-frozen until analysis. In all cases, no more than 90 minutes passed between harvest and storage in the freezer, analysis being always carried out between 24 and 48 h after subsamples were stored in the freezer. Stability of endosulfan in the matrices during the homogenization and storage procedures was previously tested on spiked samples.

Extraction of endosulfan residues from peppers, cucumbers and cherry tomatoes was carried out according to the Mills procedure^{15,16} without a Florisil clean-up step, briefly as follows:

Weigh chopped sample (100 g) into high-speed blender jar, add acetonitrile (200 ml), blend the mixture for 2 min and filter through a 12-cm Büchner funnel. Transfer the filtrate into a 1-litre separating funnel, add light petroleum (100 ml) and shake vigorously for 2 min. Add saturated sodium chloride solution (10 ml) and water (600 ml) and mix thoroughly. Discard the aqueous layer and wash the solvent layer with water (2 \times 100 ml). Filter the light petroleum layer through anhydrous sodium sulfate (10 g) and rinse the filter with light petroleum (10 ml). Evaporate the light petroleum sample extract to 1–2 ml using a vacuum rotatory evaporator (40°C water bath) and dilute to exactly 10 ml with light petroleum in a volumetric flask. In all instances it was assumed that this extract represented 10 g sample ml⁻¹. Endosulfan residues in the light petroleum extracts were determined by GC-ECD using the operating conditions described above.

3 RESULTS AND DISCUSSION

Residue data found in the degradation studies of endosulfan (α , β , sulfate and sum) on pepper, cucumber and cherry tomatoes are given in Table 1. During the first week following pesticide treatment, residue levels of α -endosulfan were, in all cases, higher than those determined for β -endosulfan, but, from day 14, residue levels found for both isomers were, in general, very similar. However, β -endosulfan seems to be slightly more persistent than α -endosulfan, which was also reported by Mukherjee and Gopal¹⁷ in a study carried out on the interconversion of stereoisomers of endosulfan on chick-pea crop under field conditions.

Endosulfan sulfate residues were first found in cucumber, pepper and tomato at days 1, 3 and 7, respectively. The maximum level of this metabolite was determined 14 days (cucumber) and 22 days (pepper and tomato) after application. These results are in agreement with those reported by Gopal and Mukherjee on the behaviour of endosulfan residues in eggplant.¹⁸

Total endosulfan residue levels (sum of α -endosulfan, β -endosulfan and endosulfan sulfate) in all the samples analysed in this study were below 1 mg kg⁻¹, this value

TABLE 1
Levels of Endosulfan (α -endosulfan, β -endosulfan, Endosulfan Sulfate and Sum) in Cucumber, Pepper and Tomato

Sample	<i>t</i> (days)	Residue (mg kg ⁻¹) ^a			
		α	β	Sulfate	Sum
Tomato	1	0.46	0.24	n.d. ^b	0.70
	3	0.29	0.24	n.d.	0.53
	7	0.23	0.19	0.04	0.46
	14	0.15	0.13	n.d.	0.28
	22	0.09	0.10	0.08	0.27
	32	0.06	0.08	0.07	0.21
	38	0.04	0.07	0.06	0.17
Cucumber	1	0.15	0.13	0.02	0.30
	3	0.08	0.06	0.11	0.25
	7	0.07	0.06	0.05	0.18
	14	0.05	0.04	0.06	0.15
	22	0.01	0.01	0.05	0.07
	32	n.d.	n.d.	0.04	0.04
	38	0.01	0.01	0.01	0.03
Pepper	1	0.30	0.09	n.d.	0.39
	3	0.17	0.09	0.07	0.33
	7	0.09	0.07	0.05	0.21
	14	0.03	0.07	0.08	0.18
	22	0.01	0.04	0.10	0.15
	32	0.01	0.02	0.06	0.09
	38	0.01	0.02	0.03	0.06

^a Mean of triplicate analyses from three replicates.

^b n.d.: not detected.

being the maximum residue limit (MRL) established by European legislation for endosulfan residues in pepper, tomato and cucumber.¹⁹

In order to evaluate the decline of total endosulfan residues as a function of time in the plantations of pepper, cucumber and tomato, and to determine the statistical parameters that describe these processes, the formal approaches proposed by Timme, Frehse and Laska¹⁴ to study the behaviour of pesticide residues on crops prior to harvest were used. According to these

authors, time and/or residue values were transformed using the six formal models described in Table 2 to achieve a linear relationship, as shown in Fig. 1. The function equation in the transformed system is always $y = a + bx$, where a is the intercept on the ordinate at $x = 0$ and b is the slope. Table 2 also shows the formulae for the decline curves in the original system (back-transformed function) for the six models.¹⁴

To select the best fit model, the modified coefficient of determination r^2 proposed by Frehse and Walter²⁰ and

TABLE 2
Coordinate Transformation in the Six Models and Formulae for the Decline Curves in the Original System (Back-Transformed Function)^a

Function	Transformation		Back-transformed function
	Ordinate (y)	Abscissa (x)	
First-order	$\log R$	None	$R = 10^{a+bt}$
1.5th-order	$1/\sqrt{R}$	None	$R = 1/(a+bt)^2$
Second-order	$1/R$	None	$R = 1/(a+bt)$
RF-first-order	$\log R$	\sqrt{t}	$R = 10^{a+b\sqrt{t}}$
RF-1.5th-order	$1/\sqrt{R}$	\sqrt{t}	$R = 1/(a+b\sqrt{t})^2$
RF-second-order	$1/R$	\sqrt{t}	$R = 1/(a+b\sqrt{t})$

^a This table has been adapted from Ref. 20.

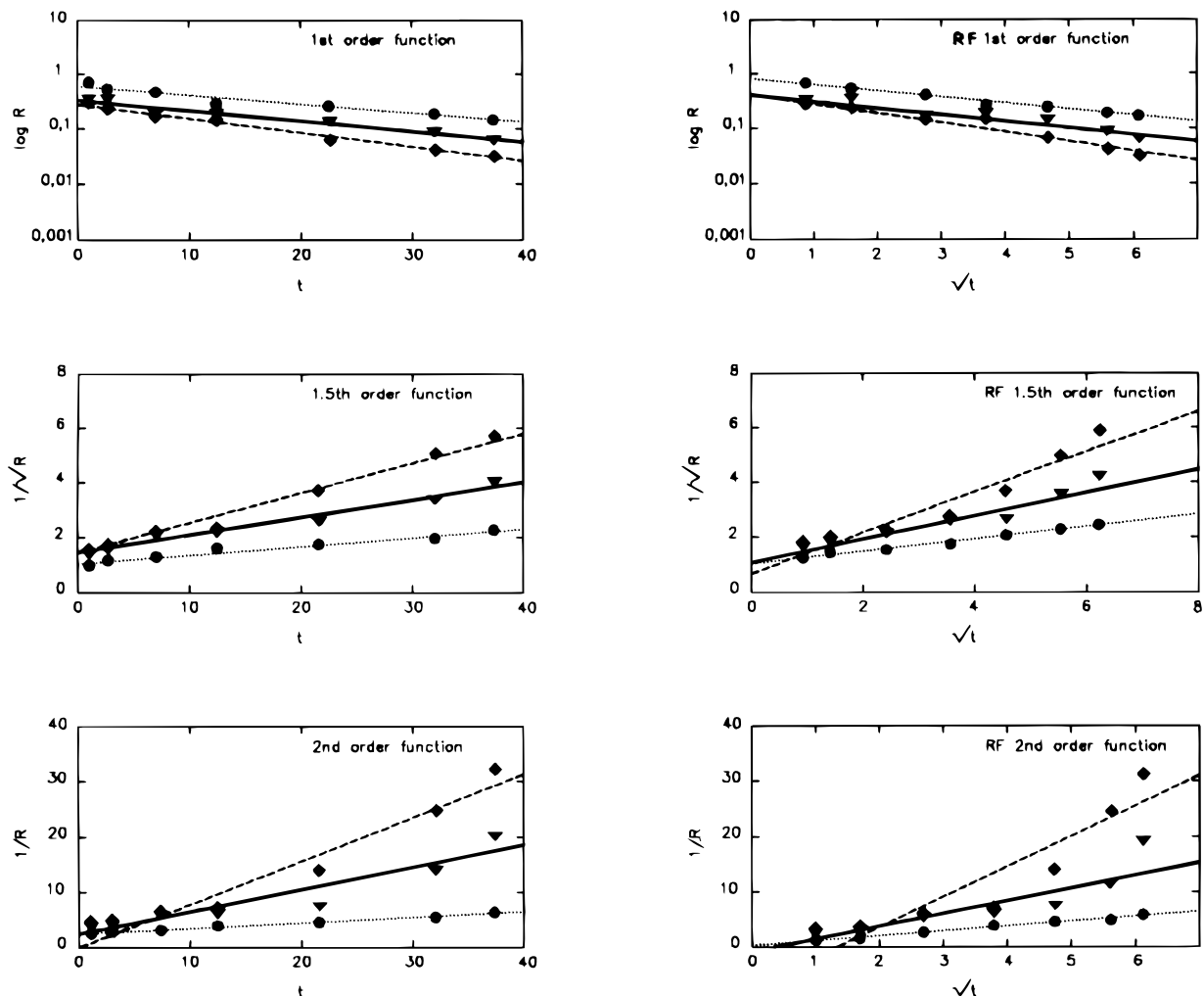


Fig. 1. Straight lines obtained for endosulfan residue data in (◆) cucumber, (▲) pepper and (●) tomato using the coordinate transformations indicated in Table 2. (t units are days and R units are mg kg^{-1})

the test quantity D described by Timme and Frehse²¹ were calculated for each model and crop. These values are indicated in Table 3, in which we can see that correlations are confirmed in all cases except for cucumber/RF 1·5-order, for which the fit was regarded as not assured since the r^2 value was negative.¹⁴ For cucumber/second-order, cucumber/RF second-order and pepper/RF second-order, the respective functions

cannot be used for the evaluation, since the constants a and b have different signs, which results in a residue value of infinity for a particular decline time.²⁰ In Fig. 2 the decline curves are plotted in the original system (back-transformed functions) for those cases in which correlation was confirmed. Although correlation was confirmed for all models plotted in Fig. 2, residue values predicted for some of these models at the beginning of

TABLE 3
Modified Coefficient of Determination (r^2) and Test Quantity for Correlation (D) Values
Determined from the Six Functions

Function	r^2 (D)		
	Cucumber	Pepper	Tomato
First-order	0·984 (0·238)	0·938 (0·214)	0·895 (0·192)
1·5th-order	0·890 (0·189)	0·963 (0·227)	0·925 (0·208)
Second-order	—	0·763 (0·119)	0·958 (0·225)
RF-first-order	0·912 (0·200)	0·971 (0·231)	0·979 (0·235)
RF-1·5th-order	—0·158	0·837 (0·160)	0·980 (0·235)
RF-second-order	—	—	0·891 (0·189)

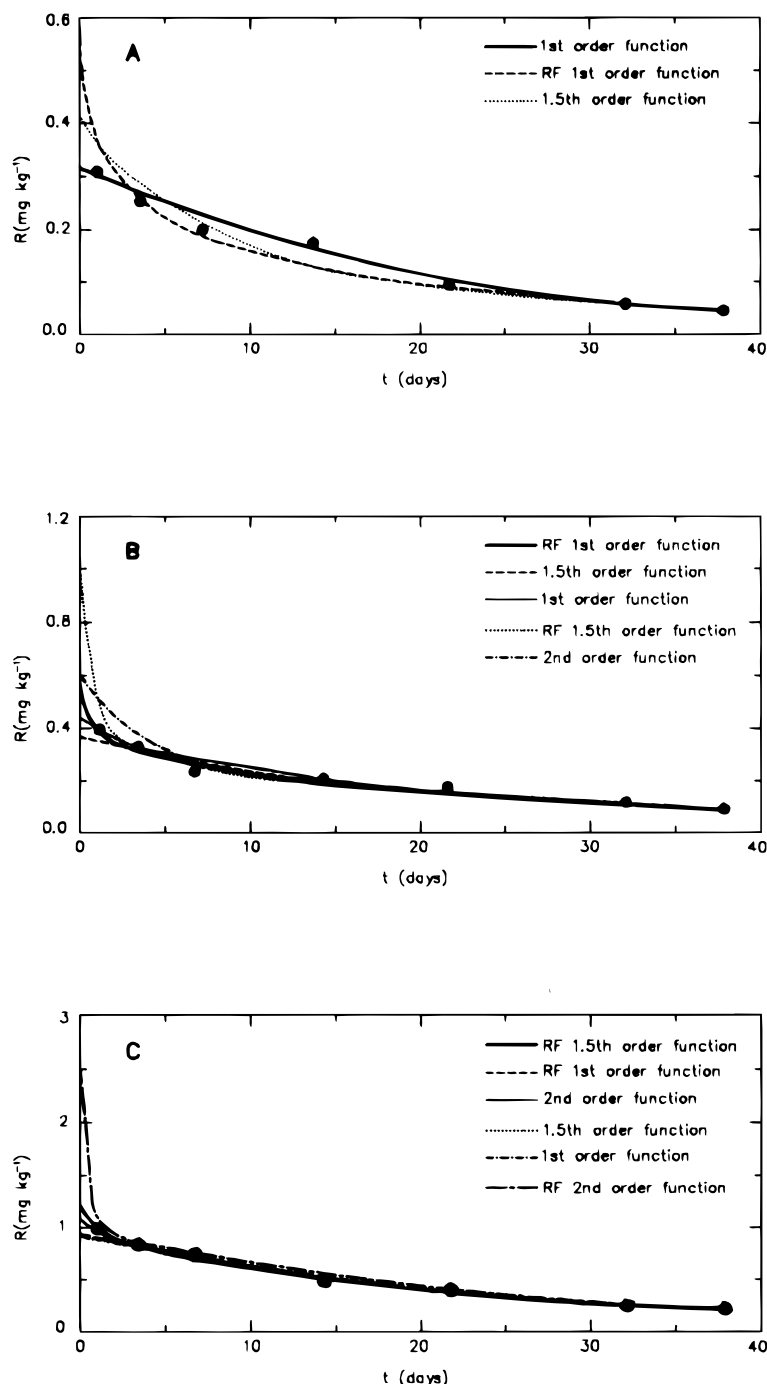


Fig. 2. Decline curves of endosulfan in (A) cucumber, (B) pepper and (C) tomato, (back-transformed function) obtained by applying the models for which correlation was confirmed. Note that full line is used for the best fit function.

the study are very different, but after three days, residue levels of endosulfan predicted for all these models are very similar and close to the experimental data.

Functions that best fit the experimental data (maximum value of r^2)¹⁴ were the first-order for cucumber, RF-first-order for pepper and RF-1.5th-order for tomato, but in this last case the 1.5th-order function gave practically the same values of r^2 and D as obtained by the best fit function. These results are in good agreement with the published data on the applicability of the six functions.²⁰

Endosulfan half-life times ($T/2$) in the three plantations determined from the first-order function and the optimal functions, by using the general formulae for decline times (T/X) derived by Timme *et al.*,^{14,20} are given in Table 4. $T/2$ values given in most studies carried out on decline of pesticide residues in crops are usually obtained assuming a pseudo-first-order reaction to describe the behaviour of residues.^{21,22} In our experiments the first-order model, although optimal for cucumber only, was confirmed in all cases, and the $T/2$ values obtained for endosulfan residues from this model

TABLE 4
Half-Life Times ($T/2$) Determined from the First-Order and the Optimal Functions

Sample	$T/2 (\pm CI) (\text{days})^a$	
	First-order function	Optimal function
Cucumber	11.1 (± 1.3)	11.1 (± 1.3) (first-order)
Pepper	15.2 (± 3.3)	8.5 (± 4.2) (first-order-RF)
Tomato	20.1 (± 6.6)	8.9 (± 4.7) (1.5th-order-RF)

^a CI: confidence interval at a significance level of $\alpha = 0.05$.

TABLE 5
Residue Values after Preharvest Interval of 15 Days

Sample	Residue (mg kg^{-1}) ($\pm CI$) ^a	
	First-order function	Optimal function
Cucumber	0.12 [0.091–0.159]	0.12 [0.091–0.159] (first-order)
Pepper	0.18 [0.125–0.267]	0.16 [0.103–0.240] (first-order-RF)
Tomato	0.35 [0.231–0.543]	0.30 [0.238–0.386] (1.5th-order-RF)

^a CI = Confidence interval at a significance level of $\alpha = 0.05$.

were 11 days (cucumber), 15 days (pepper) and 20 days (tomato), this order being similar to that of the surface/weight ratio of the vegetables. However, $T/2$ values obtained from the optimal function were similar and close to 10 days in the three plantations. It is interesting to note that for those plantations in which the first-order and the optimal function were not the same, $T/2$ values obtained from the optimal function were lower than those obtained from the first-order function. This means that in these cases, shortly after application, the residues decline at a faster rate than suggested by an assumed pseudo-first-order reaction.

Finally, endosulfan residues in the plantations after expiry of the pre-harvest interval (15 days),²³ calculated from both the first-order and the optimal function, were very similar in all cases, as can be seen in Table 5. The values obtained from the optimal function in cucumber, pepper and tomato were 0.12 mg kg^{-1} , 0.16 mg kg^{-1} and 0.30 mg kg^{-1} respectively, which correspond to 12%, 16% and 30%, respectively, of the European MRLs established for endosulfan in these crops.

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REFERENCES

1. FAO/WHO, 1967 Evaluations of some pesticide residues in food. *Food and Agriculture Organization of United Nations*, Rome, 1968.
2. Tomlin, C. (ed.), *The Pesticide Manual*. British Crop Protection Council, (Surrey, UK, The Royal Society of Chemistry Cambridge, UK), 1994, pp 388–90.
3. WHO, Endosulfan Criteria 40: Endosulfan. WHO, Geneva, 1984.
4. Schumacher, H. G., Klein, W. & Korte, F., Contributions to ecological chemistry XXXII. Photochemical reactions of endosulfan. *Tetrahedron Lett.*, **24** (1971) 2229–32.
5. Schuphan, I., Sajko, B. & Ballschmiter, K. Z., Chemical and photochemical breakdown of the cyclodiene insecticides, aldrin, dieldrin, endosulfan and other hexachlorobicyclo- (2,2,2)- hepten-derivates. *Z. Naturforsch.*, **276** (1972) 147–56.
6. Martens, R.F., Degradation of endosulfan-8,9-carbon-14 in soil under different conditions. *Bull. Environ. Contam. Toxicol.*, **17** (1977) 438–46.
7. Andersson, A. & Bergh, T., Pesticide residues in fresh fruit and vegetables on the Swedish market, January 1985–December 1989. *Fresenius' Z Anal. Chem.*, **339** (1991) 387–9.
8. Food and Drug Administration, Food and Drug Administration Pesticide Program. Residue Monitoring 1992. *J. AOAC Int.*, **76** (1993) 127A–148A.
9. Neider, E., Trotman, R. B. & Saschenbrecker, P. W., Levels and incidences of pesticide residues in selected agricultural food commodities available in Canada. *J. AOAC Int.*, **77** (1994) 18–33.
10. De Kok, A., Multiresidue methods used in the Netherlands and residue data obtained in 1993. In *Pesticide Residues' 94*, ed. A. Valverde & A. R. Fernández. IEA/UAL/COEXPHAL, Almería, 1995.
11. Chen, J. & Gao, J., The Chinese Total Diet Study in 1990. Part I. Chemical contaminants. *J. AOAC Int.*, **76** (1993) 1193–205.
12. Kan-Do Office and Pesticide Team, Accumulated pesticides and industrial chemical findings from a ten-year study of ready-to-eat foods. *J. AOAC Int.*, **78** (1995) 614–31.
13. Agüera, A., Métodos multiresiduos de análisis de pesticidas en frutas y hortalizas. Métodos de identificación.

- Doctoral Thesis*, University of Almería, 1995.
14. Timme, G., Frehse, H. & Laska, V., Statistical interpretation and graphic representation of the degradational behaviour of pesticide residues II. *Pflanzenschutz-Nach. Bayer*, **39** (1986) 187–203.
 15. Mills, P. A., Onley, J. H. & Gaither, R. A., Rapid method for chlorinated pesticide residues in nonfatty foods. *JAOC.*, **46** (1963) 186–91.
 16. Valverde Garcia, A. & Gonzalez Pradas, E., *Análisis de residuos de plaguicidas: métodos de extracción*. Instituto de Estudios Almerienses, Almería, 1989.
 17. Mukherjee, I. & Gopal, M., Interconversion of stereoisomers of endosulfan on chickpea crop under field conditions. *Pestic. Sci.*, **40** (1994) 103–6.
 18. Gopal, M. & Mukherjee, I., Determination of residues of endosulfan and endosulfan sulfate on eggplant, mustard and chickpea. *Pestic. Sci.*, **37** (1993) 67–72.
 19. Official Journal of European Communities, *Council Directive 82/528/EEC*.
 20. Frehse, H. & Walter, H. F., The behaviour of pesticide residues in fruits and vegetables: evaluation by decline curves. In *Pesticide Residues '94*, ed. A. Valverde & A. Rodriguez. IEA/UAL/COEXPHAL, Almería, 1995.
 21. Timme, G. & Frehse, H., Statistical interpretation of the degradational behaviour of pesticide residues, I. *Pflanzenschutz-Nach. Bayer*, **33** (1980) 47–60.
 22. Mukherjee, I. & Gopal, M., Residue behaviour of fenvalerate, tau-fluvalinate, lambda-cyhalothrin and monocrotophos in eggplant fruits. *Pestic. Sci.*, **36** (1992) 175–9.
 23. Liñan Y. Vicente, C., *Vademecum de productos fitosanitarios y nutricionales*. Carlos Liñan y Vicente, Madrid 1994.